The impact of vaccination on the breadth and magnitude of the antibody response to influenza A viruses in HIV-infected individuals

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Abstract: Objective: HIV-positive individuals have lower antibody titers to influenza viruses than HIV-negative individuals, and the benefits of the annual vaccinations are controversially discussed. Also, there is no information about the breadth of the antibody response in HIV-infected individuals. Design: The binding and neutralizing antibody titers to various human and nonhuman influenza A virus strain were determined in sera from 146 HIV-infected volunteers: They were compared with those found in 305 randomly selected HIV-negative donors, and put in relation to HIV-specific parameters. Uni and multivariable regression was used to identify HIV-specific parameters associated with the measured binding and neutralizing activity. Methods: Enzyme-linked immunosorbent assays and in-vitro neutralization assays were used to determine the binding and neutralizing antibodiy titers to homo and heterosubtypic influenza A subtypes. Results: We found that both homo and heterosubtypic antibody titers are lower in HIV-positive individuals. Vaccination promoted higher binding and neutralizing antibody titers to human but not to nonhuman isolates. HIV-induced immune damage (high viral load, low CD4+ T cell counts, and long untreated disease progression) is associated with impaired homosubtypic responses, but can have beneficial effects on the development of heterosubtypic antibodies, and an improved ratio of binding to neutralizing antibody titers to homosubtypic isolates. Conclusions: Our results indicate that repetitive vaccinations in HIV-positive individuals enhance antibody titers to human isolates. Interestingly, development of antibody titers to conserved heterosubtypic epitopes paradoxically appeared to profit from HIV-induced immune damage, as did the ratio of binding to neutralizing antibodies.

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Conclusions: Our results indicate that repetitive vaccinations in HIV-positive individuals enhance antibody titers to human isolates. Interestingly, development of antibody titers to conserved heterosubtypic epitopes paradoxically appeared to profit from HIV-induced immune damage, as did the ratio of binding to neutralizing antibodies.

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Keywords: antibodies, $CD4^+$ T cells, heterosubtypic, HIV, influenza, vaccination

Introduction

HIV-infected individuals have increased morbidity and mortality following influenza virus infection [1]. Current influenza virus vaccines are formulated as trivalent inoculum (TIV) and rely on inducing highly strain-specific neutralizing antibodies. Therefore, they need to be revised, reformulated, and re-applied annually. A

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pan-influenza vaccine based on strain-insensitive heterosubtypic antibodies would be desirable. It is suspected that some influenza vaccines, such as the H1/pdm09 vaccine, promote production of cross-reactive antibodies, whereas original antigenic sin (OAS), that is the preferential expansion of memory B cell clones from the initial immune response [2–4], most likely restricts their generation. We could recently show that repetitive vaccination of HIV-negative individuals generally stimulates homo and heterosubtypic responses [5].

The effectiveness of currently available influenza vaccines in HIV-positive individuals is controversially discussed. Meta-analyses estimated the effectiveness of the non-adjuvanted influenza virus vaccines in HIV-infected individuals to be only 27–78% [6], even with increased antigen dose and booster administration [7]. Nonetheless, HIV-infected children and adolescents have a significant increase in antibodies to the influenza virus strains following vaccination. Influenza virus-specific antibody titers, however, are significantly lower in HIV-positive than in HIV-negative individuals [8].

Factors contributing to the modest antibody response to TIV include both damage to the $CD4^+$ T-cell compartment and HIV-mediated alterations in the B-cell compartment. These include the impaired generation and reactivation of antigen-specific memory B cells [9], and the shrinking of the memory B cell compartment [10]. Antiretroviral therapy (ART) stops HIV disease progression, but the extent of recovery of the naïve and resting B cell compartment depends on the stage at which ART was started [11]. We therefore investigated to which degree the response to TIV depends on HIV disease-related parameters such as viral RNA load, $CD4^+$ T-cell counts, the duration of untreated HIV disease progression, and $CD4^+$ T-cell nadir, and whether repetitive vaccination helps to restore or maintain antibody titers to influenza A viruses in HIV-infected individuals.

Methods

Ethics statement and participant recruitment

All HIV-1 infected individuals enrolled in this study were cared for at the Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich and are participating in the Swiss HIV Cohort Study [12], and/or the Zurich Primary HIV Infection Study (ZPHI) [13,14]. The protocol for this study has been reviewed and approved by the ethics committee of the University Hospital Zurich and written informed consent was obtained from all participants. The Swiss HIV cohort study was approved by individual local institutional review boards of all participating centers and written informed consent was also approved for the SHCS. The ZPHI Study is approved by the local ethics board and written informed consent was obtained.

The majority of non-HIV-infected study participants have randomly been recruited from the members of the University and the University Hospital of Zurich, Switzerland. All participants completed a questionnaire to assess their influenza exposure history. After informed consent was obtained, a blood sample was drawn. Enrollment ran for the most part from 20 October, 2009 to 30 October, 2009, before the onset of the Swine Flu pandemic. Where available, data were completed with HIV-specific parameters provided by the SHCS. Our cohort comprised 83% males with a median value for age of 44 [interquartile range (IQR) 38–50], for vaccinations of 5 (IQR 2–9) and for the number of perceived influenza episodes of two (IQR 0–4).

Details on experimental and statistical methods can be found in the supplementary methods section, http://links.lww.com/QAD/A732. A comprehensive analysis of the non-HIV-infected cohort has already been published elsewhere [5].

Results

To investigate the impact of influenza vaccination and HIV-disease parameters on the breadth and magnitude of hetero and homosubtypic antibody responses in HIV-infected individuals, we collected serum from 146 randomly selected HIV-positive volunteers [most of which are also participating in the Swiss HIV Cohort Study (SHCS)] in October 2009. Using recombinant hemagglutinin (HA) proteins (c.f. supplementary methods, http://links.lww.com/QAD/A732), binding antibody titers to five human and three nonhuman influenza isolates were determined. Moreover, neutralizing antibody titers to five human and four avian influenza isolates were also measured (Fig. 1). At a serum dilution of 1 in 30, more than half of all HIV-infected donors were found to possess heterosubtypic antibodies (Fig. 1). These were then compared with epidemiological baseline characteristics, such as the number of vaccinations, the age, HIV status, and HIV-specific parameters ($CD4^+$ T-cell counts, viral load, time period before and since ART), and put in relation with data from a previous study that analyzed sera from 305 HIV-negative individuals [5].

Impact of an HIV-infection on the breadth and magnitude of the humoral antibody response to influenza A virus

As depicted in Fig. 2 and supplementary Tables S1 and S2 (http://links.lww.com/QAD/A732), HIV-infected individuals displayed lower binding titers than HIV-negative donors. In univariable analyses, binding titers to homosubtypic rH1/34 and rH3/99 viruses were lower in HIV-positive individuals compared with HIV-negative individuals. In contrast, neutralizing titers did not seem to differ between seronegative and seropositive individuals, except for H1/34 wherein higher neutralizing titers were

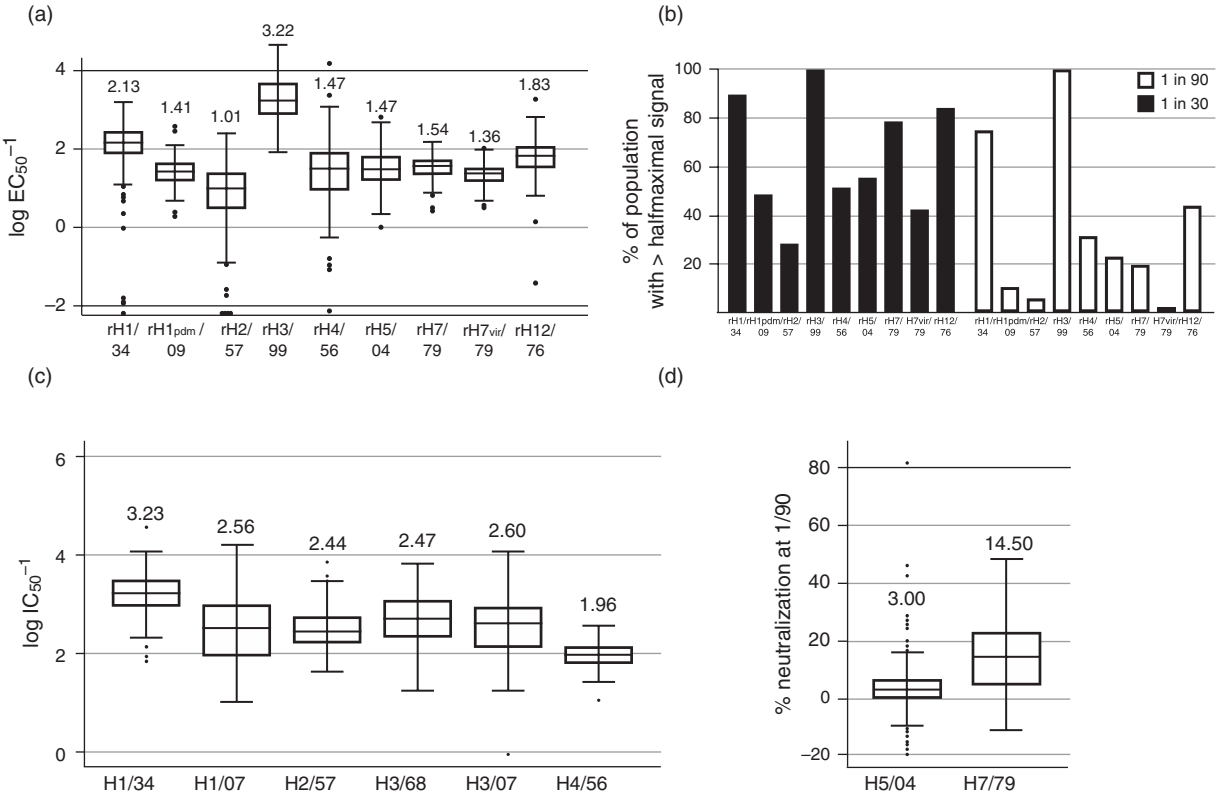

Fig. 1. Prevalence and distribution of homosubtypic and heterosubtypic serum antibodies in HIV-1-infected individuals. (a) Serum antibody reactivities against the indicated immobilized recombinant hemagglutinin were assessed by ELISA. Values are shown as the logarithm of the reciprocal serum dilution giving a half-maximal signal (log EC50). (b) Percentage of participants displaying ELISA signals that are greater than the half-maximal value against the indicated HA subtype at a serum dilution of 1 in 30 and 1 in 90. (c) Half-maximal inhibitory serum antibody titer (log IC50) to human and avian virus isolates. Values are shown as logarithm of the reciprocal serum dilution. (d) Inhibition percentage against H5/04 and H7/79 at a serum dilution of 1 in 90. Nonlinear regression could not be performed, as neutralizing titers were too low for these two subtypes. Neutralization was tested against the indicated viruses at a multiplicity of infection of 2–6 (c) and (d). Boxplots are indicating median and interquartile ranges (IQRs). Whiskers include lower or upper quartile $+1.5 \times$ IQR.

found in HIV-positive donors. Correcting for the number of vaccinations, age and sex did not change the significance of this observation (Fig. 2; supplementary Table S3, http://links.lww.com/QAD/A732). Binding titers to H7N7 virions directly coated to plates and neutralizing titers to H1/34, H3/07, H2/57, and H4/56 were comparable with those of HIV-negative donors. Neutralizing titers to H5 and H7 were too small to be fitted accurately into the Hill-equation. A surrogate value corresponding to the percentage neutralization at a serum dilution of 1 in 90 was used for the analyses. Albeit more extensive scattering, these values were also comparable between HIV-positive and HIV-negative donors.

These data indicate that an HIV infection has a more pronounced negative impact on the binding than on the neutralizing titers to human isolates; however, this difference was clearly less pronounced for H3 isolates. The neutralizing titers appeared to be less affected than the binding titers, suggesting that in HIV-infected individuals, the antibody response is more focused on neutralizing epitopes. This hypothesis is also supported by statistical examination of the fraction between the binding and the neutralizing antibody titers (supplementary Table S4, http://links.lww.com/QAD/A732) showing a better neutralizing to binding antibody titer ratio in HIV-infected individuals for most tested strains even after adjusting for the number of vaccinations.

Impact of HIV-related parameters on the breadth and magnitude of the humoral antibody response to influenza A virus

The impact of HIV-induced damage to the immune system was further evaluated using the following parameters: the last $CD4^+$ counts before the onset of ART (LCD4), the state of the $CD4^+$ compartment before the study (ICD4), the $CD4^+$ nadir (Table 1), the mean RNA levels during the year before the study (Table 2), the number of ambiguous nucleotide calls – a measure for HIV-diversity and marker for duration of infection (AMB) [15], and the duration of untreated disease progression (UTDP) following infection as measured by a

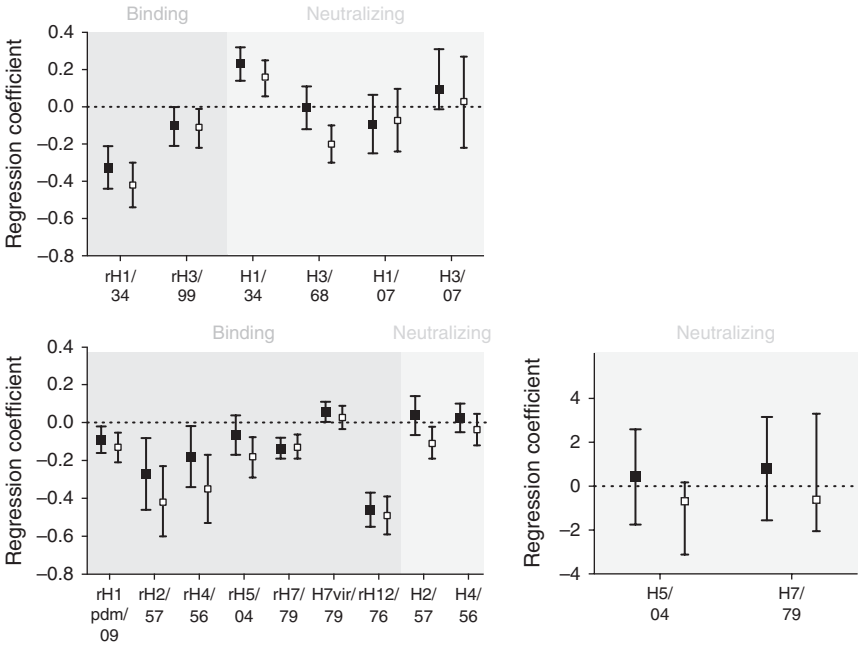

Fig. 2. Linear regression models comparing influenza titers of HIV-positive and HIV-negative populations. Univariable (solid squares) and multivariable (open squares) linear regression models comparing (a) binding and neutralizing titers to influenza homosubtypes (b) binding and neutralizing titers to heterosubtypes of HIV-negative individuals to HIV-positive individuals. Values are indicating regression coefficients; 95% confidence intervals are indicated. The same ELISA and neutralizing data as in Figure 1 were used for this analysis. A value of zero represents equal antibody titers of HIV-1 negative and HIV-1 positive individuals, positive values favor HIV-positive and negative ones HIV-negative individuals.

$CD4^+$-based statistical method developed by Taffe *et al.* [16] (UTDP; Table 3; complete analyses in supplementary Table S5, http://links.lww.com/QAD/A732).

The state of the $CD4^+$ compartment at the last measurement before the blood draw (ICD4) had no significant influence on the binding and neutralizing

Table 1. Univariable and multivariable linear regressions analyzing binding titers ($logEC_{50}$) and neutralizing titers ($logIC_{50}$) in relation with $CD4^+$ cell counts and other characteristics.

rHA		Characteristics	Regression coefficient (95% CI)	*P*
rH1/34	**Univariable**	**LCD4**	**0.77 (0.10 to 1.44)**	**0.025**
	Multivariable	**LCD4**	**0.82 (0.16 to −1.49)**	**0.015**
		Number of vaccinations	**0.037 (0.0037 to 0.070)**	**0.030**
rH1/34	**Univariable**	**ICD4**	**0.66 (0.71 to −1.25)**	**0.028**
	Multivariable	ICD4	0.57 (−0.025 to −1.17)	0.060
		Number of vaccinations	0.032 (−0.0016 to 0.065)	0.062
rH1pdm/09	**Univariable**	**ICD4**	**−0.27 (−0.51 to −0.019)**	**0.035**
	Multivariable	**ICD4**	**−0.27 (−0.51 to −0.037)**	**0.024**
		Number of vaccinations	−0.0055 (−0.019 to 0.0077)	0.410
rH5/04	**Univariable**	**ICD4**	**−0.36 (−0.70 to −0.033)**	**0.031**
	Multivariable	**ICD4**	**−0.38 (−0.72 to −0.051)**	**0.024**
		Number of vaccinations	0.00056 (−0.018 to 0.019)	0.952
rH1/34	Univariable	Nadir	1.04 (−0.026 to 2.11)	0.056
	Multivariable	**Nadir**	**1.44 (0.36 to 2.51)**	**0.010**
		Number of vaccinations	**0.041 (0.0075 to 0.074)**	**0.017**
Virus		Characteristics	Regression coefficient (95% CI)	*P*
H5/04	**Univariable**	**ICD4**	**−11.34 (−19.94 to −2.73)**	**0.010**
	Multivariable	**ICD4**	**−11.45 (−20.38 to −2.52)**	**0.012**
		Number of vaccinations	0.11 (−0.39 to 0.61)	0.662
H2/57	**Univariable**	**Nadir**	**−0.67 (−1.23 to −0.11)**	**0.019**
	Multivariable	Nadir	−0.38 (−0.84 to 0.092)	0.114
		Number of vaccinations	0.10 (−0.0039 to 0.025)	0.151

ICD4, $CD4^+$ cell count before onset of influenza study; LCD4, last $CD4^+$ cell count before onset of ART; rHAs, recombinant hemagglutinin. $CD4^+$ cell numbers are indicated per 1000. Bold values and characteristics indicate $P < 0.05$. Regressions were additionally adjusted for age and sex.

Table 2. Univariable and multivariable linear regressions analyzing binding titers ($logEC_{50}$) and neutralizing titers ($logIC_{50}$) in relation with HIV viral loads.

rHA		Characteristics	Regression coefficient (95% CI)	*P*
rH7vir/79	**Univariable**	**Mean RNA**	**0.050 (0.018 to 0.082)**	**0.003**
	Multivariable	**Mean RNA**	**0.058 (0.024 to 0.091)**	**0.001**
		Number of vaccinations	0.0016 (−0.0097 to 0.013)	0.775
Virus		Characteristics	Regression coefficient (95% CI)	*P*
H2/57	**Univariable**	**Mean RNA**	**−0.093 (−0.14 to −0.042)**	**<0.001**
	Multivariable	**Mean RNA**	**−0.055 (−0.098 to −0.012)**	**0.013**
		Number of vaccinations	0.0095 (−0.0050 to 0.024)	0.198
H3/07	**Univariable**	**Mean RNA**	**−0.18 (−0.30 to −0.060)**	**0.003**
	Multivariable	**Mean RNA**	**−0.16 (−0.29 to −0.038)**	**0.011**
		Number of vaccinations	0.0029 (−0.039 to 0.045)	0.890
H3/68	**Univariable**	**Mean RNA**	**−0.10 (−0.17 to −0.034)**	**0.003**
	Multivariable	**Mean RNA**	**−0.058 (−0.11 to −0.0020)**	**0.043**
		Number of vaccinations	0.0050 (−0.014 to 0.024)	0.597
H5/04	**Univariable**	**Mean RNA**	**1.52 (0.12 to 2.92)**	**0.033**
	Multivariable	**Mean RNA**	**1.70 (0.23 to 3.17)**	**0.024**
		Number of vaccinations	0.24 (−0.26 to 0.74)	0.342

RNA values are noted as log values (logRNA copies/ml). rHAs, recombinant hemagglutinin. Bold values and characteristics indicate $P < 0.05$. Multivariable regressions were additionally adjusted for age and sex.

antibody titers. Only neutralizing titers to H5/04 were found to decrease with lower $CD4^+$ T-cell counts. Yet, this observation partially may be an artifact because of the larger scattering of the surrogate values.

The number of $CD4^+$ cells before the initiation of ART (LCD4) did not have a major impact on binding or neutralizing antibody titers with the exception of the binding titers to H1/34 isolate, which were additionally influenced by the number of vaccinations. Similarly, the $CD4^+$ nadir also did not have a significant effect on antibody titers with the exception of binding titers to homosubtypic rH1/34, where correcting for the number of vaccinations showed an effect on both. The mean HIV

Table 3. Univariable and multivariable linear regressions analyzing binding titers ($logEC_{50}$) and neutralizing titers ($logIC_{50}$) in relation with temporal parameters and other characteristics.

rHA		Characteristics	Regression coefficient (95% CI)	*P*
rH1/34	**Univariable**	**AMB**	**−17.0048 (−31.11 to −2.90)**	**0.019**
	Multivariable	**AMB**	**−16.89 (−31.27 to −2.51)**	**0.022**
		Number of vaccinations	**0.047 (0.0038 to 0.091)**	**0.034**
rH1/34	**Univariable**	**ARTD**	**0.031 (0.0024 to 0.060)**	**0.034**
	Multivariable	ARTD	0.011 (−0.026 to 0.049)	0.543
		Number of vaccinations	0.030 (−0.011 to 0.070)	0.155
rH3/99	**Univariable**	**ARTD**	**0.029 (0.10 to 0.047)**	**0.002**
	Multivariable	**ARTD**	**0.029 (0.0062 to 0.052)**	**0.013**
		Number of vaccinations	0.011 (−0.014 to 0.036)	0.376
Virus		Characteristics	Regression coefficient (95% CI)	*P*
H1/07	**Univariable**	**ARTD**	**0.029 (0.0040 to 0.054)**	**0.023**
	Multivariable	ARTD	0.016 (−0.016 to 0.048)	0.326
		Number of vaccinations	0.010 (−0.024 to 0.045)	0.547
H3/68	**Univariable**	**ARTD**	**0.029 (0.010 to 0.048)**	**0.003**
	Multivariable	ARTD	0.0048 (−0.016 to 0.025)	0.641
		Number of vaccinations	0.0059 (−0.016 to 0.028)	0.594
H2/57	**Univariable**	**ARTD**	**0.028 (0.013 to 0.042)**	**<0.001**
	Multivariable	ARTD	0.0053 (−0.10 to 0.021)	0.508
		Number of vaccinations	0.010 (−0.0069 to 0.027)	0.204
H2/57	**Univariable**	**UTDP**	**0.026 (0.0074 to 0.045)**	**0.007**
	Multivariable	UTDP	0.0023 (−0.015 to 0.020)	0.790
		Number of vaccinations	0.012 (−0.0039 to 0.027)	0.140
H5/04	**Univariable**	**UTDP**	**0.61 (0.10 to 1.11)**	**0.019**
	Multivariable	**UTDP**	**0.61 (0.041 to 1.19)**	**0.036**
		Number of vaccinations	−0.11 (−0.63 to 0.41)	0.680

AMB, fraction of ambiguous nucleotides; ARTD, time since onset of ART till onset of influenza study ($year^{-1}$); rHAs, recombinant hemagglutinin; UTDP, untreated duration of disease progression ($year^{-1}$). Bold values and characteristics indicate $P < 0.05$. Regressions were additionally adjusted for age and sex.

RNA levels in the year before the blood sampling did have a negative impact on homosubtypic titers to H3/07, and to some extent also to H3/68 (Table 2). Moreover, binding titers to rH2/57 were also decreased when higher RNA levels were present, but this observation was confounded by age (supplementary Table S5, http://links.lww.com/QAD/A732). In contrast, high mean RNA levels correlated with increasing neutralizing activity to H5/04 at a 1 in 90 serum dilution. Similar findings were made with binding titers to purified H7 virions directly coated on plates: higher mean HIV RNA levels appeared to favor also development of more binding antibodies to avian H7. However, this improvement was also affected by being born before 1957.

The number of ambiguous nucleotide calls (AMB) [15], a marker for duration of the infection, negatively correlated with binding titers to rH1/34 (Table 3). A prolonged duration of untreated disease progression (UTDP) had a positive impact on the development of neutralizing titers to H5/04 but on no other titers. The duration of ART treatment (ARTD) only had a positive effect on binding antibodies to rH3/99, even after correcting for vaccination and age (Table 3).

Thus, in summary, there was no HIV-related parameter that could be used as a reliable predictor for the breath or magnitude of the humoral response to influenza A viruses. Quite interestingly though is the observation that in cases wherein an impact of the HIV-induced damage to the immune system was detected, it tended to be negative for human, but positive for avian nonhuman isolates.

Impact of influenza vaccination in HIV-infected individuals

Next, when the impact of vaccination on homo- and heterosubtypic antibody titers was analyzed (supplementary Table S6, http://links.lww.com/QAD/A732), it became apparent that the number of vaccinations yielded no significant improvement in neutralizing titers to the strains included in the last vaccine applied before the blood donation (i.e., H1/09 and H3/07). Yet, the number of vaccinations clearly improved binding and neutralizing titers to the older influenza virus isolates H1/34 and H3/68. For latter, being born before 1957 was found to be a confounding factor in the multivariate analysis (supplementary Table S6, http://links.lww.com/QAD/A732). This is probably because of an epitope shared between rH1pdm/09 and H1N1 viruses circulating before 1957 [17].

With respect to heterosubtypic responses, vaccination or age had no impact on either binding or neutralizing titers. Binding and neutralizing titers to H2/57 were significantly better in individuals born before 1968, thus those who actually could have been infected with this subtype. It was therefore not surprising to find confounding of age for all H2/57-specific antibody titers. When adjusting the impact of vaccination on heterosubtypic binding titers by age (supplementary Table S6, http://links.lww.com/QAD/A732), we additionally found that in participants born between 1928 and 1957, binding titers to rH1pdm/09 were also positively associated with increasing age.

It can therefore be deducted that although the response to recently applied strains was moderate, vaccination helped to maintain titers to older isolates. It was therefore partially able to undo/prevent deterioration of these titers following HIV infection.

Discussion

For the first time, in addition to human influenza A viruses, we also included avian influenza A viruses and assessed the impact of an HIV-infection on binding and neutralizing antibody titers. We could show that all HIV-positive donors bore homosubtypic and half of them also heterosubtypic serum antibodies, albeit at lower titers than HIV-negative donors. Vaccination increased homosubtypic but had no impact on heterosubtypic antibody titers, which is in contrast to HIV-negative donors. Viral load, $CD4^+$ T-cell counts, and antiviral treatment were found as predictors for some binding and neutralizing antibodies to both human and nonhuman subtypes.

The generally lower binding titers found in HIV-positive donors against humans and avian viruses compare well to observations made in other studies investigating pre-vaccination sera [18], and probably are the result of the poorer vaccine response in HIV-positive individuals [8]. However, using inverted H7-proteins in ELISA, we could demonstrate that also in HIV-positive donors, most of the heterosubtypic antibodies recognized conserved eptiopes in the stem of the HA protein (supplementary Figure S1, http://links.lww.com/QAD/A732).

As the memory B cell compartment remains compromised under ART [19], we hypothesized that the antibody response to influenza vaccination would be less affected by OAS-related limitations. However, this was not the case, as we found stronger binding and neutralizing antibody responses to older than to more recent strains in frequently vaccinated HIV-positive individuals. Thus, HIV-induced damage to the immune system clearly impairs the vaccine response to homosubtypic viruses.

In contrast to binding titers, only small differences in neutralizing titers between HIV-positive and HIV-negative individuals were found. More frequent vaccination of the HIV-positive group is likely to account for these minor differences. Interestingly, however, was the finding that the difference between binding and neutralizing titers (log EC50 – log IC50) was much lower in HIV-infected (supplementary Table S4, http://links.lww.com/QAD/

A732) when compared with HIV-negative individuals. This was most prominent for the old H1/34 isolate wherein HIV-infected individuals clearly produced less non-neutralizing, HA-binding antibodies than uninfected donors, but showed similar titers of neutralizing antibodies. One may speculate that the immune damage induced by HIV preferentially depleted the immune system of B cells with biologically irrelevant antibody specificities, which would normally compete with more specific and biologically relevant antibodies.

Association of HIV-specific parameters ($CD4^+$ T-cell counts, viral loads and duration of infection) with binding and neutralizing antibody titers identified $CD4^+$ T-cell counts as positive predictor for homosubtypic and as negative predictor for heterosubtypic antibodies. Thus, in HIV-infected individuals, $CD4^+$ T cells appear beneficial for antibody responses to homosubtypic viruses but disadvantageous for heterosubtypic antibody responses.

Previous studies either identified the number of $CD4^+$ T-cells as a direct determinant of the antibody response to influenza vaccination [18,20], or could not show correlations between influenza serum antibody titers and $CD4^+$ T-cell counts in HIV-infected subjects [8]. Besides low $CD4^+$ T-cell numbers, high viral loads and prolonged duration of untreated disease progression favored the generation of heterosubtypic antibodies. Thus, HIV-induced damage to the immune system seems to favor the generation of heterosubtypic antibodies. One might speculate that a decimation of overrepresented homosubtypic memory B cells allows for less abundant heterosubtypic B cells to expand without heavy competition by abundant heterosubtypic B cells.

In contrast, binding and neutralizing antibody titers to both heterosubtypic and homosubtypic viruses increased with ART treatment duration. This may be explained by a strong association of the duration of ART treatment and the number of vaccinations. Although all other HIV parameters showed contrary results for heterosubtypes and homosubtypes, treatment duration equally improved titers to both homosubtypic and heterosubtypic viruses in response to vaccination.

Although the overall picture from these observations is intriguing and logical, it has to be mentioned that the individual significance levels for the HIV parameter-related analyses are not very strong, and also the pattern of significances could be more consistent.

Nonetheless, our results clearly support the importance of vaccination of HIV-infected individuals against influenza viruses. To promote an immune response in HIV-positive individuals that also produces antibodies to heterosubtypic avian strains, vaccine designs need to be improved to induce antibodies directed against conserved epitopes be enclosed in both human homosubtypic and heterosubtypic strains and in avian heterosubtypic strains.

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I.K. planned and performed the majority of the experiments. R.K. performed all statistical analyses together with I.K. Experimental work was assisted by M.B. and A.W. C.G. was the study nurse who collected all blood samples used for this study. Prof. Dr med. Huldrych Günthard was the accompanying physician and holder of the ethical permit. Prof. Günthard was also crucially contributing to the planning and analysis of the study. Prof. Lars Hangartner was the principal investigator who initiated and conducted this study in collaboration with the aforementioned other contributors.

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Conflicts of interest

The authors report no conflicts of interest.

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